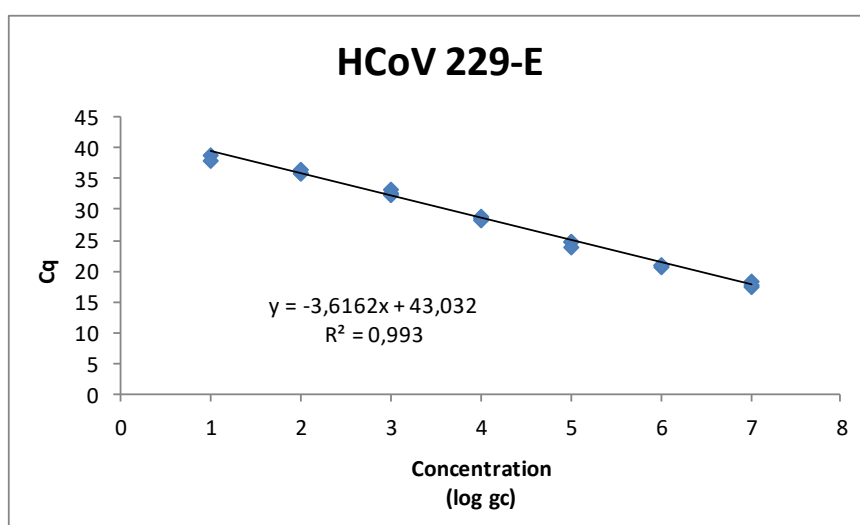


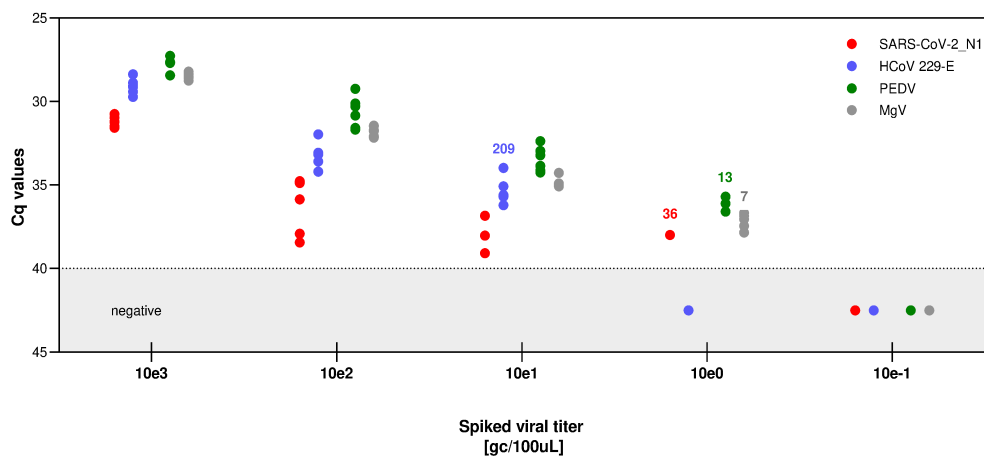
**Supplementary Material**

*Primers and probe, reaction mix, thermal cycling conditions and calibration curve used to detect and quantify HCoV 229-E.*

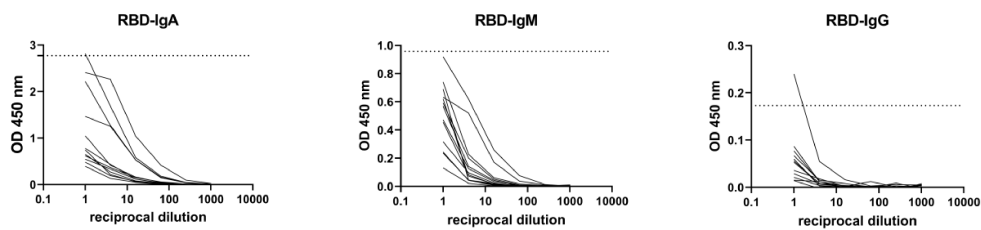
Reaction mix (10  $\mu$ L) consisted of 5.00  $\mu$ L 2X One Step RT-PCR Buffer III, 0.20  $\mu$ L PrimeScript RT enzyme Mix II, 0.20  $\mu$ L ROX, 0.20 mL TaKaRa Ex Taq HS, 0.30  $\mu$ L 229E-F and 229E-R primers (10mM), 0.15  $\mu$ L 229E-P probe (10mM). The cycling parameters were as RT at 48 °C for 30 min, preheating at 95 °C for 10 min and 45 cycles of amplification at 95 °C for 15 s, and 60 °C for 1 min.



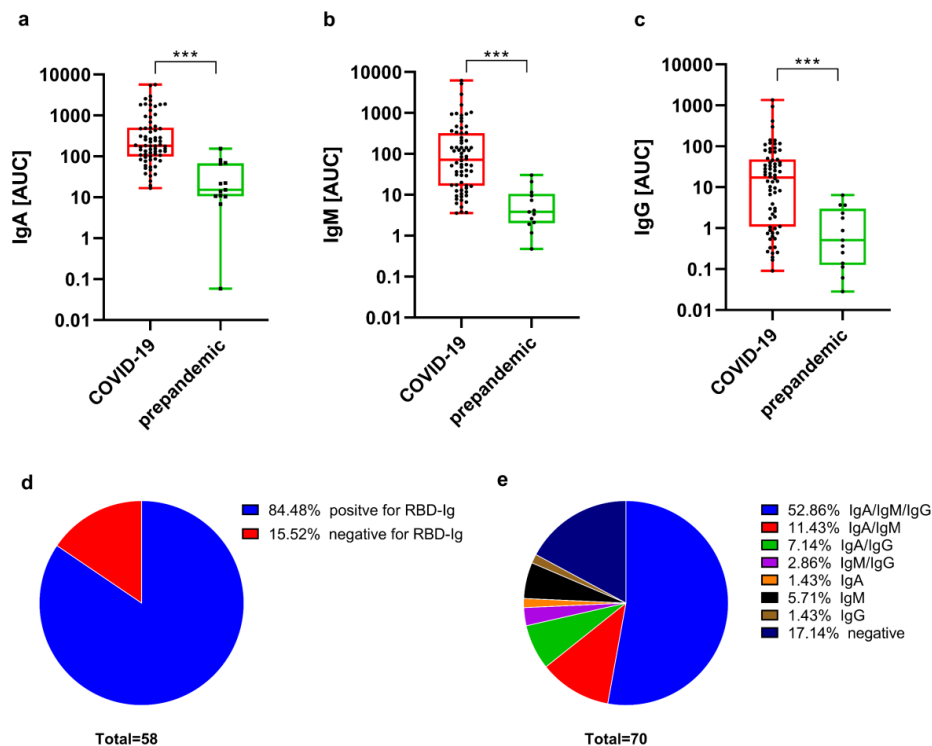
**Figure S1. Standard curve generated to quantify HCoV 229-E performed with 10-fold dilutions ( $10^0$ - $10^7$  gc/reaction) of genomic RNA.**



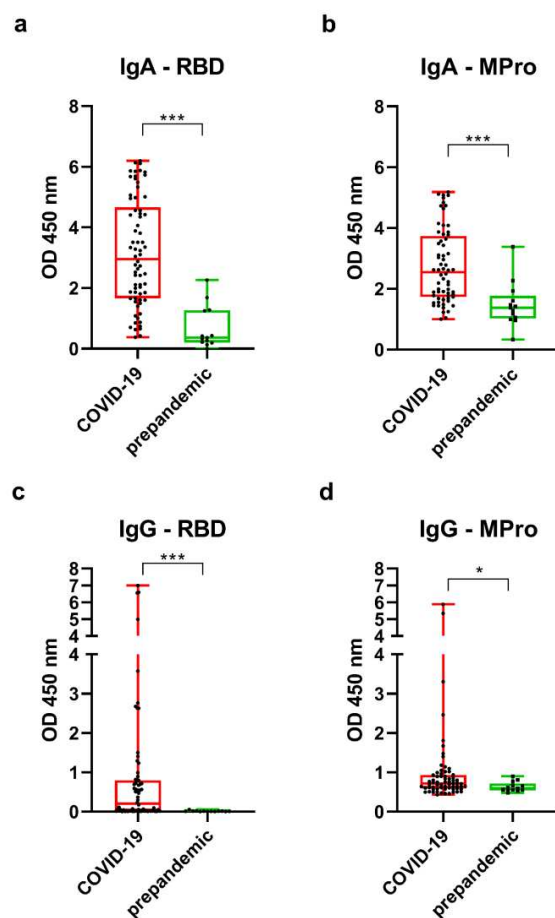
**Figure S2. Distribution of cycle threshold values (Cq) characterizing the limits of detection of the automated assisted method (Max) to extract viral RNA form breast milk samples:** severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human coronavirus 229E (HCoV 229-E), porcine epidemic diarrhea virus (PEDV), and mengovirus (MgV) spiked in breast milk. The numbers above viral series denotes the  $LoD_{95\%}$  (gc/100uL) for each virus.



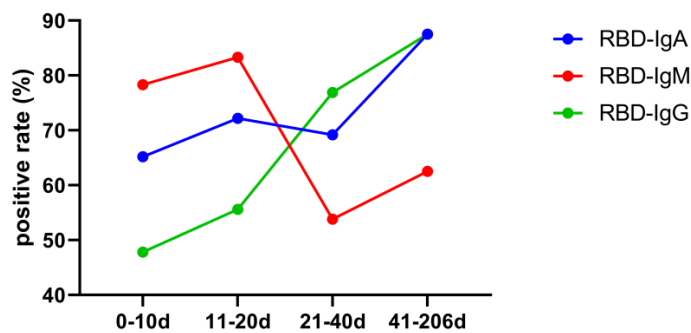
**Figure S3: Titration curves of prepandemic Control samples.** Serial dilutions of prepandemic Controls to determine cut-off values in order to discriminate between negative and positive samples in COVID-19 collected breastmilk samples, calculated as the mean + 2SD.



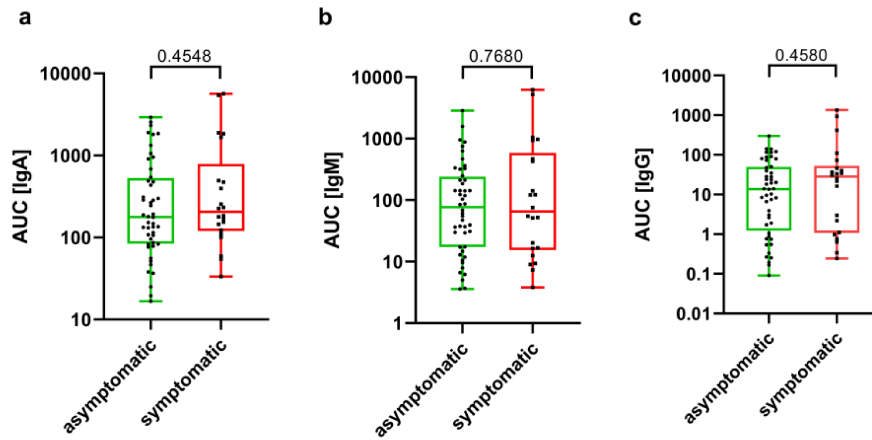
**Figure S4: Breast milk from COVID-19 infected and/or recovered mothers show significantly higher antibody binding to the RBD antigen of SARS-CoV-2 than prepandemic controls and are detectable in 82.9 % of samples.** AUC were calculated from titration curves for RBD-reactive (a) IgA, (b) IgM, and (c) IgG in order to get a better graphical impression. Asterisks show statistically significant differences between groups (\*\*\*p < 0.0001) using the Mann–Whitney test (unpaired nonparametric test). (d) Proportion of human milk donors who had positive RBD-reactive Igs in at least one milk recollection point, and negative samples (e) Proportion of RBD-reactive positive and negative human milk samples subdivided according to different isotypes.



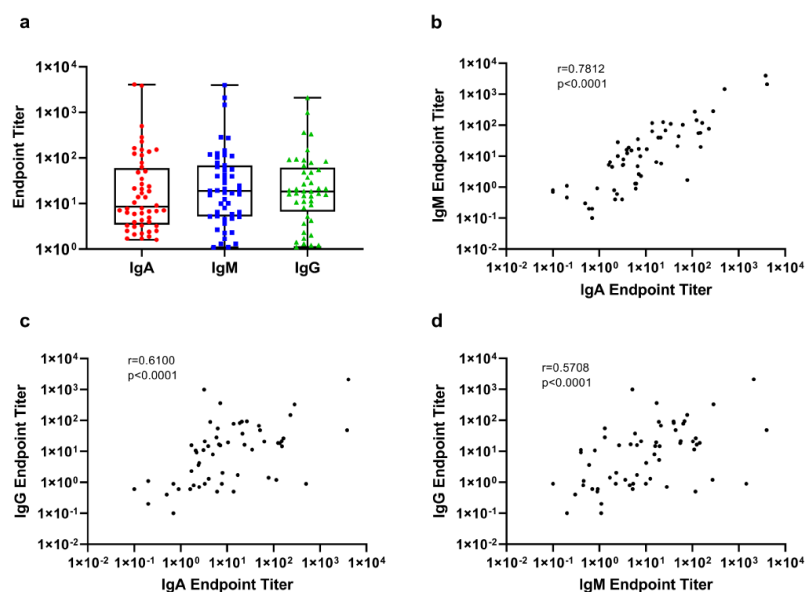
**Figure S5: Samples from infected milk donors show significantly higher binding to SARS-CoV-2 antigens RBD and Mpro compared to prepandemic control.** Grouped OD values of 1:4 diluted samples of RBD- (a) and MPro- (b) reactive IgA, and RBD- (c) and MPro- (d) reactive IgG, respectively. Asterisks show statistically significant differences between groups (\*\* $p < 0.0001$ , \* $p < 0.05$ ) using the Mann-Whitney test (unpaired nonparametric test).



**Figure S6:** Graph of positive rates of RBD-specific IgA, IgM and IgG versus days after positive PCR diagnosis.

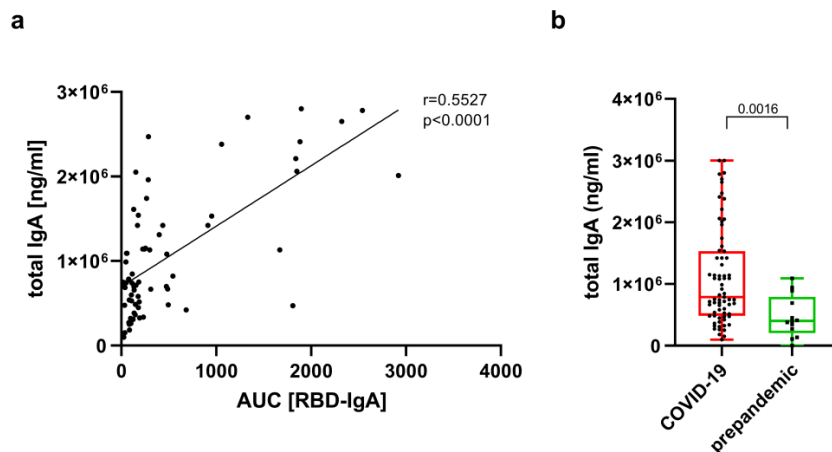


**Figure S7: : Reactivity of breast milk samples from asymptomatic and symptomatic COVID-19 infected and/or recovered mothers to RBD antigen.** AUC were calculated from titration curves for RBD-reactive (a) IgA, (b) IgM and (c) IgG in order to get a better graphical impression. Mann–Whitney test (unpaired nonparametric test) was used to assess for statistical significance.



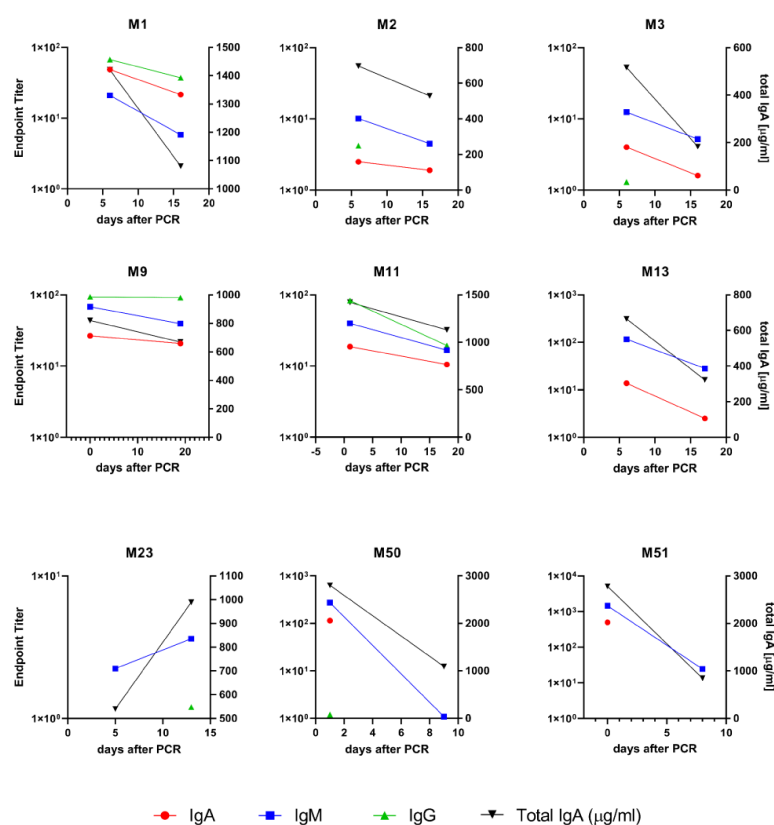
**Figure S8: Endpoint titers for RBD-specific IgA, IgM and IgG and correlation analysis between antibody subclasses.** (a) Grouped endpoint titers of the three different isotypes. Spearmans' correlation analysis of endpoint titers between (a) RBD-specific IgA and RBD-IgM, (b) RBD-IgA and RBD-IgG, and (c) RBD-IgM and RBD-IgG.



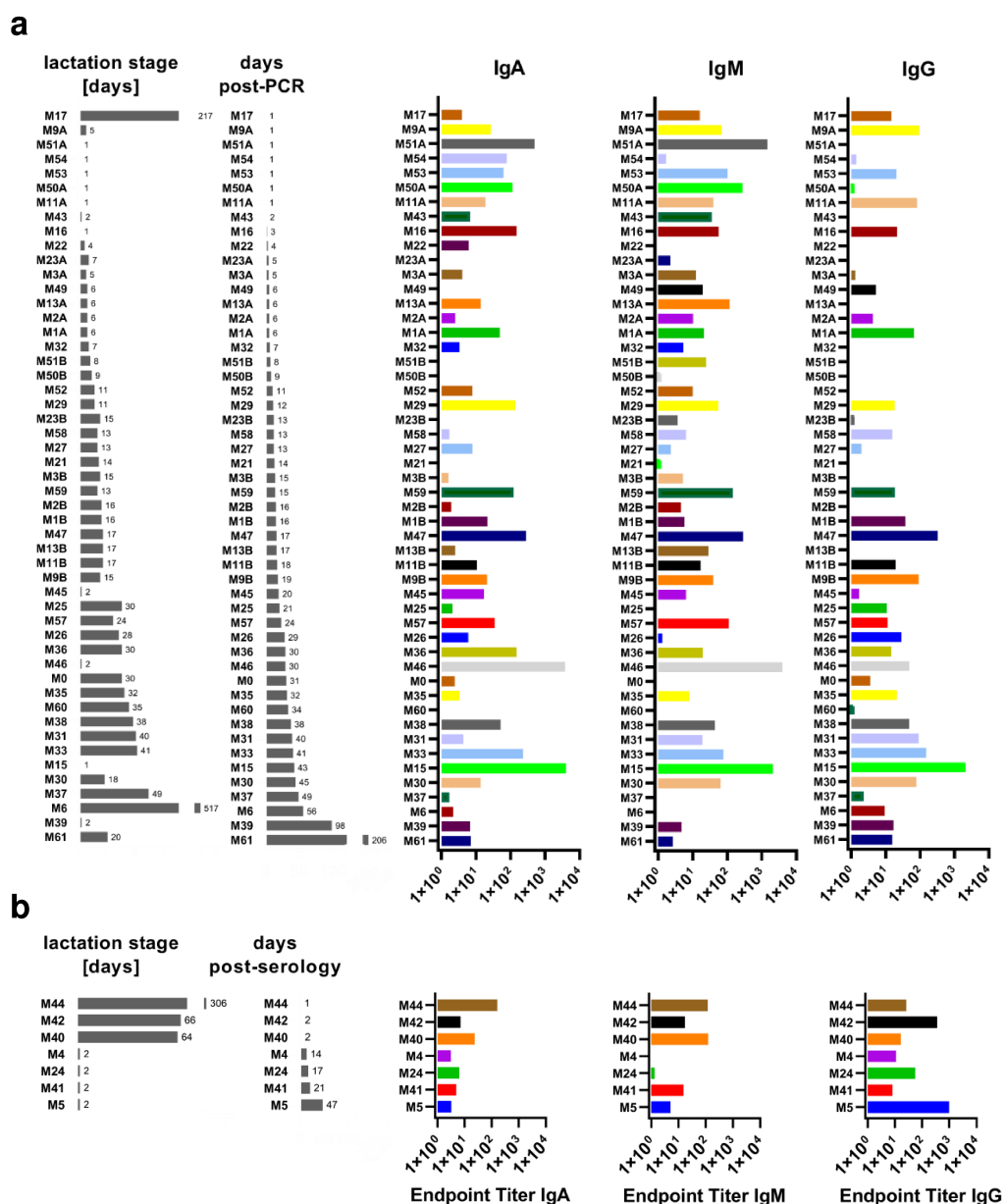


**Figure S9: Total IgA concentration and virus specific antibody response.**

(a) Spearman's correlation analysis of total IgA concentration and virus specific IgA response expressed as AUC. (b) Total IgA in COVID-19 infected and recovered and prepandemic control samples. Mann–Whitney test (unpaired nonparametric test) was used to assess for statistical significance.



**Figure S10: Temporal dynamic changes of endpoint titers in longitudinal samples for RBD-specific Igs.** Longitudinal samples at two time points from 12 mothers were available, samples from 3 mothers were negative at both times and not represented. Virus-specific IgA (red circles), IgM (blue squares), IgG (green triangles) and total IgA (black triangles). All isotypes were tested for RBD binding in both time points, only positive endpoint titer are drawn in the graphs, the absence of data in a given time point indicates samples that were below the Cut-off values and considered negative.



**Figure S11: Individual endpoint titers of virus-specific antibodies in breast milk from SARS-CoV-2 infected and/or recovered mothers.** (a) Endpoint titers of milk samples tested positive at least in one of the isotypes IgA, IgM and IgG are shown, ordered as days post-PCR. (B) Endpoint titers of milk samples tested positive at least in one of the isotypes IgA, IgM, and IgG are shown, ordered as days post-serology.